Understanding RNH1-mediated translation specificity:

Differences in the translation machinery of hematopoietic and non-hematopoietic tissue

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1. Abstract

The Ribonucelase Inhibitor 1 (RNH1) has been discovered to have a crucial role in the translation mechanism in higher vertebrate species. It has been shown, that RNH1-deficiency leads to decease due to a defective translation in the erythroid lineage, while other tissues are not affected by this translation defect. Ribosomopathies, a group of entities all originating from mutations regarding ribosomal proteins (RPs) also show a mostly hematopoietic-specific phenotype, building a bridge between RNH1 and ribosomopathies. Angiogenin (ANG) is a ribonuclease and is supected to play a role in the compensatory mechanism for the impenetrance of RNH1-deficiency in non-hematopoietic tissue. This work was intened to contribute data, that could help better understand the mechanism(s) behind *RNH1-mediated translation specificity*. Experiments on different levels of translation were perforemed. The results revealed, that ANG indeed is capable of ameliorating the RNH1-mediated specific phenotype and thereby may thus be involved in a compensatory mechanism in non-hematopoietic tissues.

2. Introduction

Ribosomopathies are a heterogenous group of acquired and inherited malignancies which are caused by haploinsufficiency of RPs or faulty ribosomal subunit assembly [1]. Despite the ubiquitous demand for functioning ribosomes in every tissue type, symptoms are mostly related to impaired erythropoiesis [2].

RNH1 is a cytoplasmic ribonuclease (RNase) inhibiting protein and it was shown to play an important role in the process of translation [3]. Interestingly, it was just recently discovered that RNH1-knockout (KO) mice died as a result of severe impairment of erythropoiesis, while non-hematopoietic tissue types remained unaffected [4]. This is due to an unexpectedly important role of RNH1 in the regulation of GATA1 translation, GATA1 being the most important erythroid transcription factor [5]. In the absence of RNH1, GATA1 protein levels are markedly decreased, leading to lethal impairment of erythropoiesis while GATA1-mRNA transcription is not altered (Fig. 1) [4].
Bringing the picture together, it was shown that RP-mutations can result in GATA1- and RNH1-protein deficiency, yet, their mRNA transcription is not impaired [6, 7]. Knowing that RNH1-deficiency leads to severe erythroid aplasia, a potential vitious cycle is created that could be responsible for the erythropoietic tissue specific phenotype.

ANG, an RNase, is expressed in every tissue except hematopoietic cells. It has promoting and inhibiting properties in translation and its function is governed by RNH1 [9]. This nominates ANG as an interesting participant protein in recovering *RNH1-mediated translation specificity* in erythropoiesis.

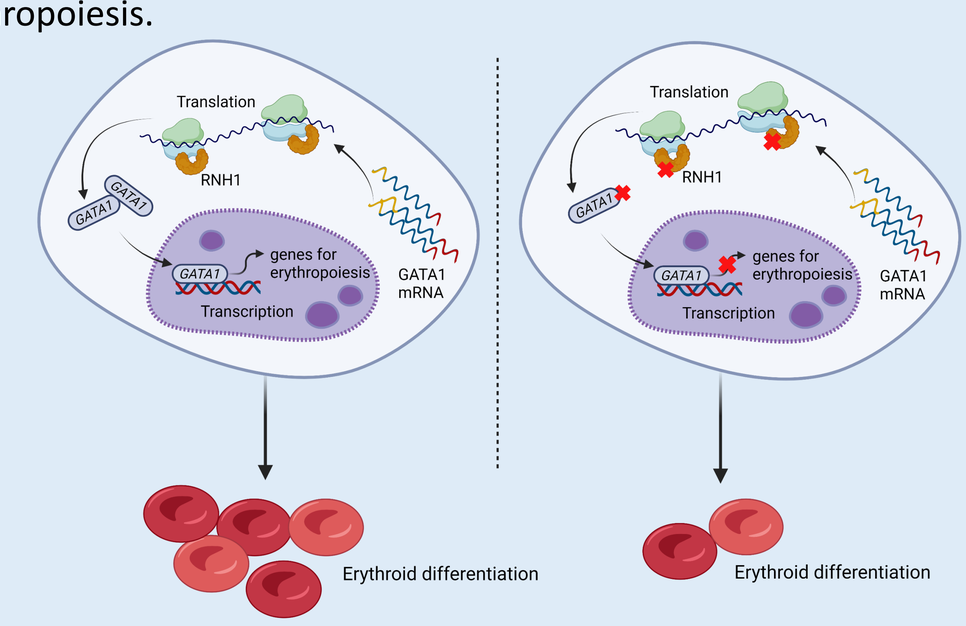

Figure 1: The mechanism of RNH1-mediated translation specificity (Chennupati et al., 2018, p. 2 – adapted)

3. Aim & leading Question

The aim of this project was to assess the role of ANG in the process of translation in hematopoietic and non-hematopoietic cell lines, inter alia.

- Is ANG capable of ameliorating the RNH1-mediated translation defect in hematopoietic and non-hematopoietic cells?

4. Methods & Material

To evaluate the possible role of ANG in RNH1-deficient circumstances, HeLa and HaCaT wildtype (WT) and RNH1-KO cells (*non-hematopoietic*), and K562 WT and K562 RNH1-KO and K562 RNH1-KO ANG-overexpressed cells (*hematopoietic*) were generated by gateway cloned plasmids and CRISPR-Cas9 in advance by other lab members.
To assess the capability of ANG to recover the RNH1-mediated translation defect in K562-KO cells, the protein labeling with O-propargyl-puromycin (OPP) combined with the Click-Chemistry™ -reaction was used. OPP is incorporated in active translation of the cells and subsequently labeled with a fluorophore. By that, global fluorescence intensity can be linked to active translation in a quantitative manner.
Western-Blots (WB), a technique to visualize and display selected proteins in a cell repertoire semiquantitatively, was used to observe the expression of ANG-protein in RNH1-deficient circumstances in the cell lines HeLa and HaCaT.

5. Results

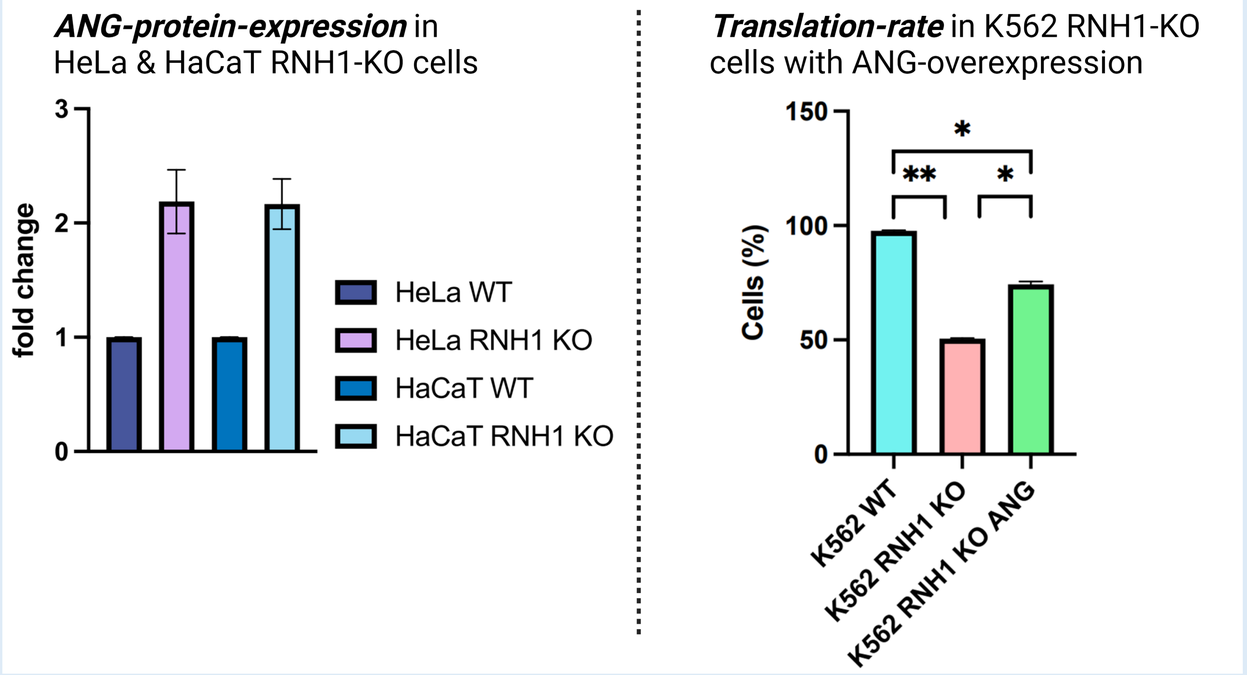

Figure 2: Results for WB-analysis and the OPP-experiment. **Left)** Western-Blot quantifiaction of ANG in the non-hematopoietic HeLa and HaCaT WT & RNH1-KO cell lines. **Right)** Bar graphs of the OPP-experiment to asses the effect of ANG-overexpression in the absence of RNH1 in the hematopoietic erythroleukaemia cell line K562 (* = P <0.03; ** = P <0.006 (α = 0.05)) (Greub, 2023)

6. Discussion & Conclusion

Interestingly, it was in fact possible to show a major role for ANG in rescuing the RNH1-mediated translational defect. By introducing ANG into the human erythroleukemia cell line K562, it was possible to cure the translational defect of RNH1-KO cells by half. Further corroborating of this conclusion was provided by WB which revealed that the concentration of ANG protein was markedly increased by a two-fold change in HeLa and HaCaT RNH1-KO cells compared to the WT-clone.

It is truly a great discovery, that ANG is indeed able to ameliorate the translational defect of hematopoietic cells and the overexpression of ANG in non-hematopoietic cells in the absence of RNH1 further strengthens the claim. Unraveling the exact molecular mechanism(s) can open new doors for treatment of ribosomopathies, anemia and possibly lead to a deeper understanding of translation in eukaryotes.

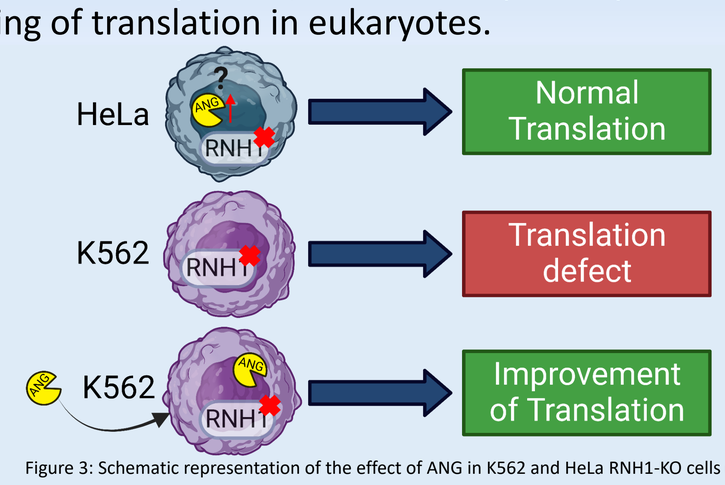

Figure 3: Schematic representation of the effect of ANG in K562 and HeLa RNH1-KO cells (Greub, 2023)

List of references:
[1] Narla, A., & Ebert, B. L. (2010). Ribosomopathies: Human disorders ofribosome dysfunction. *Blood*, 115(16), 3196–3205. https://doi.org/10.1182/blood-2009-10-178129
[2] Mills, E. W., & Green, R. (2017). Ribosomopathies: There's strength in numbers. *Science (New York, N.Y.)*, 358(6363), 2755. https://doi.org/10.1126/science.aan2755
[3] Haigis et al., 2002, p. 962.
[4] Chennupati et al., 2018, p. 2.
[5] Gutiérrez, L., Caballero, N., Fernández-Calleja, L., Karkoulia, E., & Strouboulis, J. (2020). Regulation of GATA1 levels in erythropoiesis. *IUBMB Life*, 72(1), 89–105. https://doi.org/10.1002/iub.2192
[6] Khajuria et al., 2018, p. 97.
[7] Ludwig et al., 2014, p. 749.
[9] Su et al., 2019, p. 16936.

List of figures:
Figure 1: *The mechanism of RNH1-mediated translation specificity*. Chennupati et al., 2018, p 17.
Figure 2: Greub, F. (2023). *Results for WB-analysis and the OPP-experiment*. medi.
Figure 3: Greub, F. (2023). *Schematic representation of the effect of ANG in K562 and HeLa RNH1-KO cells*. medi.